



SEQUENCE LISTING

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<110> Kimoto, Norihiro
Yamamoto, Hiroaki
Mitsubishi, Kazuya

<120> NOVEL CARBONYL REDUCTASE, METHOD FOR PRODUCING SAID ENZYME, DNA
ENCODING SAID ENZYME, AND METHOD FOR PRODUCING ALCOHOL USING SAID
ENZYME

<130> 06501-050001

<140> US 09/468,738

<141> 1999-12-21

<150> JP 1999-171160

<151> 1999-06-17

<150> JP 1998-363130

<151> 1998-12-21

<160> 29

<170> PatentIn Ver. 2.0, reformatted using WordPerfect 5.1

<210> 1

<211> 879

<212> DNA

<213> Kluyveromyces aestuarii

<400> 1

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| atgacgtttc | agcatttttt | aagaggtgga | ttagaagata | aaacagttcc | tcaggagcca | 60 |
| ccgaaggagc | aatatcccga | tggtgttaat | tacttgagct | tggtcagtc | gaaagggaaa | 120 |
| ttgacagtta | tacttggttg | agcaggagcc | attggcggag | ctctgtgtga | gggatttgcg | 180 |
| tcctgtggat | ctgacgttgt | catttttagat | tacaaatata | gtcctgaatt | gtcatcagtt | 240 |
| ttggaatcta | ggtatggagt | gaggtcgaaa | agctatcagg | tcgacattac | gagttcagaa | 300 |
| gacgtgaaac | ttgttggtgc | aaagatttta | gaagattttc | ctgatcgcca | tatcaatata | 360 |
| tttgttgcta | atgcagggtat | tgcatggacc | aacggttcca | ttttgaacga | aaacgcgacg | 420 |
| ccagatgtgt | ggaaacgtgt | tatggatgtg | aacgtgcaag | gaacttatca | ttgtgcgaaa | 480 |
| tatgtggcag | aagtgttcaa | acaacagggc | catggtaatc | tgattttgac | tgcgctcgatg | 540 |
| tcaagttata | taagcaacgt | tccaactac | caaacatgtt | ataatgcctc | taaagcggcc | 600 |
| gtcagacata | tggcaaaggg | atttgctgtt | gaattcgccc | atttgacaaa | ccccgcaggt | 660 |
| aaaatcagat | gcaattcggg | ttcacctggg | tacactgaca | ccgcactttc | agcttttggt | 720 |
| ccggtcgaac | agcgcgctca | gtggtgggga | ttgactccta | tggtgcgcca | agcattacca | 780 |
| caagagctag | tcggagccta | cttgattttg | gcactctgacg | ctgcatcatt | cacaaatgga | 840 |
| tgtgatattc | aagtagacgg | tgggtacact | tgcgtttga | | | 879 |

<210> 2

<211> 292

<212> PRT

<213> Kluyveromyces aestuarii

<400> 2

| | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Met | Thr | Phe | Gln | His | Phe | Leu | Arg | Gly | Gly | Leu | Glu | Asp | Lys | Thr | Val |
| 1 | | | | 5 | | | | 10 | | | | | 15 | | |
| Pro | Gln | Glu | Pro | Pro | Lys | Glu | Gln | Tyr | Pro | Asp | Gly | Val | Asn | Tyr | Leu |

[illegible]

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<210> 3
<211> 10
<212> PRT
<213> Kluyveromyces aestuarii
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<400> 3
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  1             5             10
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<210> 4
<211> 10
<212> PRT
<213> Kluyveromyces aestuarii
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<400> 4
Tyr Ser Pro Glu Leu Ser Ser Val Leu Glu
  1                      5              10
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<210> 5
<211> 10
<212> PRT
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<213> Kluyveromyces aestuarii

<400> 5

Gly Phe Ala Val Glu Phe Ala His Leu Thr
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<210> 6

<211> 35

<212> DNA

<213> Artificial Sequence

<220>

<223> Artificially Synthesized Primer Sequence

<400> 6

gacggatcca cwttycarca yttyytragr ggwgg

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<210> 7

<211> 35

<212> DNA

<213> Artificial Sequence

<220>

<223> Artificially Synthesized Primer Sequence

<400> 7

gtgaagcttc cwccyctyar raartgytgr aawgt

35

<210> 8

<211> 38

<212> DNA

<213> Artificial Sequence

<220>

<223> Artificially Synthesized Primer Sequence

<400> 8

gacggatcct aytstccwga rttrtsttst gtwtrga

38

<210> 9

<211> 38

<212> DNA

<213> Artificial Sequence

<220>

<223> Artificially Synthesized Primer Sequence

<400> 9

gtgaagcttt cyaawacasa asayaaytcw ggasarta

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<211> 32

<212> DNA

<213> Artificial Sequence

<220>

<223> Artificially Synthesized Primer Sequence

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<221> misc_feature
<222> (0)...(0)
<223> n = A, T, G, or C

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<210> 11
<211> 32
<212> DNA
<213> Artificial Sequence

<220>
<223> Artificially Synthesized Primer Sequence

<221> misc_feature
<222> (0)...(0)
<223> n = A, T, G, or C

<400> 11
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<210> 12
<211> 254
<212> DNA
<213> Kluyveromyces aestuarii

<400> 12
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ccaccgaagg agcaatatcc cgatggtggt aattacttga gcttgttcag tcagaaaggg 120
aaattgacag ttatcactgg tggagcagga gccattggcg gagctctgtg tgagggattt 180
gcgtcctgtg gatctgacgt tgtcatttta gattacaaat actctcctga attatcttct 240
gtattagaaa gctt 254

<210> 13
<211> 650
<212> DNA
<213> Kluyveromyces aestuarii

<400> 13
ggatccactt ttcagcactt tctgagggga ggattagaag ataaaacagt tcctcaggag 60
ccaccgaagg agcaatatcc cgatggtggt aattacttga gcttgttcag tcagaaaggg 120
aaattgacag ttatcactgg tggagcagga gccattggcg gagctctgtg tgagggattt 180
gcgtcctgtg gatctgacgt tgtcatttta gattacaaat acagtcctga attgtcatca 240
gttttggaat ctaggtatgg agtgaggtcg aaaagctatc aggtcgacat tacgagttca 300
gaagacgtga aacttgttgt tgcaaagatt ttagaagatt ttcctgatcg cgatatcaat 360
acatttggtg ctaatgcagg tattgcatgg accaacggtt ccattttgaa cgaaaacgcg 420
acgccagatg tgtggaaacg tgttatggat gtgaacgtgc aaggaaactta tcattgtgcg 480
aaatatgtgg cagaagtgtt caaacaacag ggccatggta atctgatttt gactgcgtcg 540
atgtcaagtt atataagcaa cgttcccaac taccaaacat gttataatgc ctctaaagcg 600
gccgtcagac atatggcaaa gggttttgca gtagagttcg cacaagctt 650

<210> 14
<211> 437
<212> DNA
<213> Kluyveromyces aestuarii

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<400> 14
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 agctatcagg tcgacattac gagttcagaa gacgtgaaac ttgtttgtgc aaagatttta 120
 gaagattttc ctgatcgcca tatcaatata tttgttgcta atgcaggat tgcatggacc 180
 aacggttcca ttttgaacga aaacgcgacg ccagatgtgt ggaaacgtgt tatggatgtg 240
 aacgtgcaag gaacttatca ttgtgcgaaa tatgtggcag aagtgttcaa acaacagggc 300
 catggtaatc tgattttgac tgcgtcgatg tcaagttata taagcaacgt tcccaactac 360
 caaacatgtt ataatgcctc taaagcggcc gtcagacata tggcaaaggg attcgcagta 420
 gaattcgctc aaagctt 437

<210> 15
 <211> 19
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Artificially Synthesized Primer Sequence

<400> 15
 tcggtggctc ctgaggaac 19

<210> 16
 <211> 23
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Artificially Synthesized Primer Sequence

<400> 16
 acatgttata atgcctctaa agc 23

<210> 17
 <211> 1787
 <212> DNA
 <213> Kluyveromyces aestuarii

<221> misc_feature
 <222> (0)...(0)
 <223> n = A, T, G, or C

<400> 17
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 gcacwtwmcy tmaaaamgaw atmgtatgrg gkrtsacgt acacawygta ttwagttygc 120
 maagacaagg cgraatagat gacgracgtt ggctgtaaat gtcgggggac naaatagatg 180
 caaataawts wgmnaymwww gkmmymkwyn ttttttaa atgcctggta actacggcag 240
 catgggctcg gtggtaggga aagaacaatt agtctatatt taggagagag gtataaataa 300
 atgaaaagat gcatatggaa attggataat ttcacacaat ttacgatgga ctgatctgta 360
 catgaactct ttgatatgta tcttatgtta tttttccttt aagcgacttc atagtggttt 420
 cgggcctcgt tcatcgga gtagctttg cacctgagtt tgggtttaga cacactataa 480
 gaagagttaa aagtctagga agtattcaaa aaataaagta aaagtcgcaa tgacgtttca 540
 gcatttttta agaggtggat tagaagataa aacagttcct caggagccac cgaaggagca 600
 atatcccgat ggtgttaatt acttgagctt gttcagtcag aaagggaaat tgacagttat 660
 cactgggtgga gcaggagcca ttggcggagc tctgtgtgag ggatttgcgt cctgtggatc 720
 tgacgttgct atttttagatt acaaatagac tcctgaattg tcatcagttt tgggaatctag 780
 gtatggagtg aggtcgaaaa gctatcaggt cgacattacg agttcagaag acgtgaaact 840

| | | | | | | |
|------------|-------------|-------------|------------|------------|------------|------|
| tggtgttgca | aagatttttag | aagatttttcc | tgatcgcgat | atcaatacat | ttgttgctaa | 900 |
| tgcaggtatt | gcatggacca | acggttccat | tttgaacgaa | aacgcgacgc | cagatgtgtg | 960 |
| gaaacgtgtt | atggatgtga | acgtgcaagg | aacttatcat | tgtgcgaaat | atgtggcaga | 1020 |
| agtgttcaaa | caacagggcc | atggtaatct | gattttgact | gcgtcgatgt | caagttatat | 1080 |
| aagcaacggt | cccaactacc | aaacatgtta | taatgcctct | aaagcggccg | tcagacatat | 1140 |
| ggcaaaggga | tttgctgttg | aattcgccca | tttgacaaac | cccgcaggta | aaatcagatg | 1200 |
| caattcggtt | tcacctggtt | acactgacac | cgcactttca | gcttttggtc | cggtcgaaca | 1260 |
| gcgcgctcag | tggtggggat | tgactcctat | gggtcgcgaa | gcattaccac | aagagcyagt | 1320 |
| cggagcctac | ttgtatttgg | catctgacgc | tgcatcattc | acaaatggat | gtgatattca | 1380 |
| agtagacggt | gggtacactt | gcgtttgatt | ttgaacaaat | ccaaatcaac | ggttatttac | 1440 |
| cgttatanaa | tttggctama | agtwttanca | natnyancga | attattcama | rawmwtttyc | 1500 |
| ccccanagtt | gcyymymyc | ycaaraatga | cmttgттаaw | mmsywtgtwr | aaacacggca | 1560 |
| gttyccyatg | gtattgattc | gatgagttac | aactttcatc | acgtaaaaat | gccagaaaaa | 1620 |
| aaaaggttgt | taatcaaata | gacacgcttt | cgaaaccatg | catcaaacgg | tcctaaacag | 1680 |
| aacaatacag | gacactgtgc | agcatgggtat | cggcaatcca | aagcttcata | tccacaagac | 1740 |
| tcatcaacga | agtcactccc | agtcacattt | tttggattta | tgcgatt | | 1787 |

<210> 18

<211> 36

<212> DNA

<213> Artificial Sequence

<220>

<223> Artificially Synthesized Primer Sequence

<400> 18

| | | | | |
|------------|------------|------------|--------|----|
| tcaggatcca | acaatgactt | ttcagcattt | tttaag | 36 |
|------------|------------|------------|--------|----|

<210> 19

<211> 31

<212> DNA

<213> Artificial Sequence

<220>

<223> Artificially Synthesized Primer Sequence

<400> 19

| | | | | |
|------------|------------|------------|---|----|
| tggtctagat | taaacgcaag | tgtacccacc | g | 31 |
|------------|------------|------------|---|----|

<210> 20

<211> 23

<212> DNA

<213> Artificial Sequence

<220>

<223> Artificially Synthesized Primer Sequence

<400> 20

| | | | |
|------------|------------|-----|----|
| tctgtatcag | gctgaaaatc | ttc | 23 |
|------------|------------|-----|----|

<210> 21

<211> 29

<212> DNA

<213> Artificial Sequence

<220>

<223> Artificially Synthesized Primer Sequence

<400> 21
atatattaat gtatcgatta aataaggag

29

<210> 22
<211> 891
<212> DNA
<213> Kluyveromyces aestuarii

<400> 22
atggatccaa caatgacggt tcagcatttt ttaagagggt gattagaaga taaaacagtt 60
cctcaggagc caccgaagga gcaatatccc gatggtgtta attacttgag cttgttcagt 120
cagaaagggg aattgacagt tatcactggt ggagcaggag ccattggcgg agctctgtgt 180
gagggatttg cgtcctgtgg atctgacggt gtcatttttag attacaaata cagtcttgaa 240
ttgtcatcag ttttggaaatc taggtatgga gtgagggtcga aaagctatca ggtcgacatt 300
acgagttcag aagacgtgaa acttgtttgtt gcaaagattt tagaagattt tcctgatcgc 360
gatatcaata ctttgtttgc taatgcaggt attgcatgga ccaacgggtc ctttttgaac 420
gaaaacgcga cgccagatgt gtggaaacgt gttatggatg tgaacgtgca aggaacttat 480
cattgtgcga aatatgtggc agaagtgttc aaacaacagg gccatggtaa tctgattttg 540
actgcgtcga tgtcaagtta tataagcaac gttcccaact accaaacatg ttataatgcc 600
tctaaagcgg cgtcagaca tatggcaaag ggatttgcgt ttgaattcgc ccatttgaca 660
aaccgcgcag gtaaaatcag atgcaattcg gtttcacctg gttacactga caccgcactt 720
tcagcttttg ttccggtcga acagcgcgct cagtgggtggg gattgactcc tatgggtcgc 780
gaagcattac cacaagagct agtcggagcc tacttgtatt tggcatctga cgctgcatca 840
ttcacaaatg gatgtgatat tcaagtagac ggtgggtaca cttgcgttta a 891

<210> 23
<211> 296
<212> PRT
<213> Kluyveromyces aestuarii

<400> 23
Met Asp Pro Thr Met Thr Phe Gln His Phe Leu Arg Gly Gly Leu Glu
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Asp Lys Thr Val Pro Gln Glu Pro Pro Lys Glu Gln Tyr Pro Asp Gly
20 25 30
Val Asn Tyr Leu Ser Leu Phe Ser Gln Lys Gly Lys Leu Thr Val Ile
35 40 45
Thr Gly Gly Ala Gly Ala Ile Gly Gly Ala Leu Cys Glu Gly Phe Ala
50 55 60
Ser Cys Gly Ser Asp Val Val Ile Leu Asp Tyr Lys Tyr Ser Pro Glu
65 70 75 80
Leu Ser Ser Val Leu Glu Ser Arg Tyr Gly Val Arg Ser Lys Ser Tyr
85 90 95
Gln Val Asp Ile Thr Ser Ser Glu Asp Val Lys Leu Val Val Ala Lys
100 105 110
Ile Leu Glu Asp Phe Pro Asp Arg Asp Ile Asn Thr Phe Val Ala Asn
115 120 125
Ala Gly Ile Ala Trp Thr Asn Gly Ser Ile Leu Asn Glu Asn Ala Thr
130 135 140
Pro Asp Val Trp Lys Arg Val Met Asp Val Asn Val Gln Gly Thr Tyr
145 150 155 160
His Cys Ala Lys Tyr Val Ala Glu Val Phe Lys Gln Gln Gly His Gly
165 170 175
Asn Leu Ile Leu Thr Ala Ser Met Ser Ser Tyr Ile Ser Asn Val Pro
180 185 190
Asn Tyr Gln Thr Cys Tyr Asn Ala Ser Lys Ala Ala Val Arg His Met

| | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| | 195 | | 200 | | 205 | | | | | | | | | | |
| Ala | Lys | Gly | Phe | Ala | Val | Glu | Phe | Ala | His | Leu | Thr | Asn | Pro | Ala | Gly |
| | 210 | | | | | 215 | | | | | 220 | | | | |
| Lys | Ile | Arg | Cys | Asn | Ser | Val | Ser | Pro | Gly | Tyr | Thr | Asp | Thr | Ala | Leu |
| 225 | | | | | 230 | | | | | 235 | | | | | 240 |
| Ser | Ala | Phe | Val | Pro | Val | Glu | Gln | Arg | Ala | Gln | Trp | Trp | Gly | Leu | Thr |
| | | | 245 | | | | | | 250 | | | | | 255 | |
| Pro | Met | Gly | Arg | Glu | Ala | Leu | Pro | Gln | Glu | Leu | Val | Gly | Ala | Tyr | Leu |
| | | 260 | | | | | | 265 | | | | | 270 | | |
| Tyr | Leu | Ala | Ser | Asp | Ala | Ala | Ser | Phe | Thr | Asn | Gly | Cys | Asp | Ile | Gln |
| | 275 | | | | | 280 | | | | | 285 | | | | |
| Val | Asp | Gly | Gly | Tyr | Thr | Cys | Val | | | | | | | | |
| | 290 | | | | | 295 | | | | | | | | | |

<210> 24
 <211> 31
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Artificially Synthesized Primer Sequence

<400> 24
 aattctcgag taatctagag gaattctaaa a 31

<210> 25
 <211> 31
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Artificially Synthesized Primer Sequence

<400> 25
 ctagtttttag aattcctcta gattactoga g 31

<210> 26
 <211> 35
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Artificially Synthesized Primer Sequence

<400> 26
 gaggaattca tacatgtatc cagatttaaa aggaa 35

<210> 27
 <211> 30
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Artificially Synthesized Primer Sequence

<400> 27
 ggtaagcttt cattaaccgc ggcttgctg 30

<210> 28
 <211> 786
 <212> DNA
 <213> *Bacillus subtilis*

<400> 28
 atgtatccag atttaaaagg aaaagtcgtc gctattacag gagctgcttc agggctcgga 60
 aaggcgatgg ccattcgctt cggcaaggag caggcaaaag tggttatcaa ctattatagt 120
 aataaacaag atccgaacga ggtaaaagaa gaggtcatca aggcgggagg tgaagctggt 180
 gtcgtccaag gagatgtcac gaaagaggaa gatgtaaaaa atatcgtgca aacggcaatt 240
 aaggagttcg gcacactcga tattatgatt aataatgccg gtcttgaaaa tctgtgcca 300
 tctcacgaaa tgccgctcaa ggattgggat aaagtcacg gcacgaactt aacgggtgcc 360
 tttttaggaa gccgtgaagc gattaaatat ttcgtagaaa acgatatcaa gggaaatgtc 420
 attaacatgt ccagtgtgca cgaagtgatt ccttggccgt tatttgtcca ctatgcggca 480
 agtaaaggcg ggataaagct gatgacagaa acattagcgt tggaatacgc gccgaagggc 540
 attcgcgtca ataattattg gccagggtgc atcaacacgc caatcaatgc tgaaaaattc 600
 gctgacccta aacagaaaagc tgatgtagaa agcatgattc caatgggata tatcggcgaa 660
 ccggaggaga tcgccgcagt agcagcctgg cttgcttcga aggaagccag ctacgtcaca 720
 ggcacacgt tattcgcgga cggcggtatg acacaatatc cttcattcca ggcaggccgc 780
 gggttaa 786

<210> 29
 <211> 261
 <212> PRT
 <213> *Bacillus subtilis*

<400> 29
 Met Tyr Pro Asp Leu Lys Gly Lys Val Val Ala Ile Thr Gly Ala Ala
 1 5 10 15
 Ser Gly Leu Gly Lys Ala Met Ala Ile Arg Phe Gly Lys Glu Gln Ala
 20 25 30
 Lys Val Val Ile Asn Tyr Tyr Ser Asn Lys Gln Asp Pro Asn Glu Val
 35 40 45
 Lys Glu Glu Val Ile Lys Ala Gly Gly Glu Ala Val Val Val Gln Gly
 50 55 60
 Asp Val Thr Lys Glu Glu Asp Val Lys Asn Ile Val Gln Thr Ala Ile
 65 70 75 80
 Lys Glu Phe Gly Thr Leu Asp Ile Met Ile Asn Asn Ala Gly Leu Glu
 85 90 95
 Asn Pro Val Pro Ser His Glu Met Pro Leu Lys Asp Trp Asp Lys Val
 100 105 110
 Ile Gly Thr Asn Leu Thr Gly Ala Phe Leu Gly Ser Arg Glu Ala Ile
 115 120 125
 Lys Tyr Phe Val Glu Asn Asp Ile Lys Gly Asn Val Ile Asn Met Ser
 130 135 140
 Ser Val His Glu Val Ile Pro Trp Pro Leu Phe Val His Tyr Ala Ala
 145 150 155 160
 Ser Lys Gly Gly Ile Lys Leu Met Thr Glu Thr Leu Ala Leu Glu Tyr
 165 170 175
 Ala Pro Lys Gly Ile Arg Val Asn Asn Ile Gly Pro Gly Ala Ile Asn
 180 185 190
 Thr Pro Ile Asn Ala Glu Lys Phe Ala Asp Pro Lys Gln Lys Ala Asp
 195 200 205
 Val Glu Ser Met Ile Pro Met Gly Tyr Ile Gly Glu Pro Glu Glu Ile
 210 215 220
 Ala Ala Val Ala Ala Trp Leu Ala Ser Lys Glu Ala Ser Tyr Val Thr

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